Determination of Ethyl Mercury and Methyl Mercury in Blood Samples

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Abstract: Thiomersal is a mercury-containing organic compound, commonly used as a preservative in topical pharmaceutical preparations, cosmetics, and biological products such as vaccines, as well as a disinfectant during dialysis treatment. The purpose of the present work was to develop a sensitive and accurate method for determination of ethyl mercury (EtHg) and methyl mercury (MeHg) in whole blood of dialysis patients.

Key words: ethyl mercury, methyl mercury, blood, speciation

INTRODUCTION

Current mercury investigations have focused primarily on MeHg from fish contamination and from rare occupational or catastrophic events. Recently, another source of exposure has been identified. Thiomersal, a preservative with both bactericidal and fungicidal action utilized in the production of biological and pharmaceutical products, contains 49.6 % EtHg by weight. [1] Because of an increasing awareness of the theoretical potential for neurotoxicity of even low levels of organomercurials, development of analytical techniques for determination of low concentrations of EtHg and MeHg is required.

RESULTS AND DISCUSSION

Metal speciation is impossible without the use of modern hyphenated techniques, in which highly sensitive and selective elemental detection systems are coupled to modern chromatographic separation systems. One of the most widely used separation method is gas chromatography which however requires volatile species. The main disadvantage of the commonly used derivatization reagent sodium tetraethylborate ($NaEt_4B$) is that the important ethylmercury species cannot be distinguished from inorganic Hg after ethylation. [2] In this work sodium tetra(n-propyl)borate ($NaPr_4B$) was tested for simultaneous determination of EtHg and MeHg in blood samples.

The method proposed is based on acid leaching (5 % H_2SO_4/18 % KBr/1M $CuSO_4$), extraction of EtHgBr and MeHgBr into an organic solvent (CH_2Cl_2), followed by back extraction into Milli-Q water, subsequent propylation with a 1 % solution of $NaPr_4B$, room temperature precollection on Tenax, isothermal gas chromatographic separation

(80 °C), pyrolysis (600 °C) and cold vapour atomic fluorescence spectrometric detection (CV AFS). [3,4]

Optimization of the method was performed on a number of different blood samples of patients before and after dialysis treatment. The concentrations of MeHg were comparable to concentrations of MeHg in the normal healthy population and showed similar values before and after dialysis. The concentration of EtHg were much elevated after treatment and reached up to 4 ng/g. Some of the results are shown in Table 1.

Table 1. MeHg and EtHg results in blood samples obtained using $NaPr_4B$ as a derivatization regent.

Sample		EtHg (as Hg) ng/g	MeHg (as Hg) ng/g
1	BT	0.09±0.00	0.36±0.04
	AT	1.74±0.07	0.33±0.05
2	BT	1.69±0.00	0.20±0.01
	AT	4.23±0.12	0.23±0.02
3	BT	0.05±0.01	0.15±0.02
	AT	1.54±0.14	0.11±0.01
4	BT	<0.01	0.18±0.01
	AT	1.06±0.08	0.17±0.01

BT- before dialysis treatment; AT - after dialysis treatment

The performance of $NaEt_4B$ and $NaPr_4B$ as derivatization reagents was checked. Comparison of the results in Table 2 shows that the values for MeHg obtained by the two derivatization reagents are in good agreement.

Table 2. Comparison of MeHg results obtained by $NaEt_4B$ and $NaPr_4B$ as derivatization reagents.

Sample	MeHg (as Hg) ng/g	
	$NaPr_4B$	$NaEt_4B$
1	0.45±0.01	0.47±0.01
2	1.13±0.04	0.92±0.05
CRM IAEA 405	5.26±0.49	5.02

IAEA 405 Estuarine sediment: certified value 4.96-6.02 ng/g

The limit of detection calculated on the basis of three times the standard deviation of the repeatability of the results was about 5-10 % for EtHg and 5-15 % for MeHg. Recoveries were between 90-110 % for both species. A certified reference material was tested to check the accuracy of MeHg determination, but for EtHg no CRM was available.

CONCLUSIONS

The analytical procedure developed was found to be a suitable and appropriate method for determination of low concentrations of EtHg and MeHg in blood samples. It also shows great potential for determination of both species in other biological samples influenced by thiomersal.

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